

08. Juni 1989
I 8896-EP/PCT wi

CLAIMS

1. HIV-3 retrovirus or variants of this virus having the essential morphological and immunological properties of any of the retroviruses deposited at the European Collection of Animal Cell Cultures (ECACC) under N° V88060301.
2. The purified retrovirus of claim 1, characterized in that said essential morphological and immunological properties are as follows:
- The virus exhibits a tropism for T4 lymphocytes.
 - The virus is cytotoxic for the lymphocytes that it infects.
 - The virus has a diameter of approximately 120 nm.
 - The virus possesses a magnesium dependent reverse transcriptase activity.
 - It can be cultivated in T4 receptor-bearing immortalized cell lines.
 - Lysates of the virus contain a p25 protein which is immunologically distinct from the p19 protein of HTLV-I by Western blot analysis.
 - Lysates of the virus contain a gp120 protein which is immunologically distinct from the gp110 protein of HTLV-I by Western blot analysis.
 - The lysate of the virus contains in addition a glycoprotein with a molecular weight of 40,000 - 45,000.
 - The genomic RNA of HIV-3 hybridizes neither with the sequences of HIV-1 nor with the sequences of HIV-2 under stringent hybridization conditions.

1

3. The retrovirus of claim 1 or 2, characterized in that the nucleotide sequence of its genomic RNA which comprises an R region and an U3 region also comprises a nucleotide sequence corresponding with the following nucleotide sequence:

5

	10	20	30	40	50	60
	OCCATGGATT TGAAGATACA CATAAAGAAA TACTGATGTG GAAGTTTGAT AGATCTCTAG					
10	70	80	90	100	110	120
	GCAACACCCA TGTTCCTATG ATAACCTCAC CAGAGCTCTT CCAGAAGGAC TAAAAACTGC					
	130	140	150	160	170	180
	TGACCTGAAG ATTGCTGACA CTGTGGAAC TCCAGCAA GACTGCTGAC ACTGCGGGGA					
	190	200	210	220	230	240
	CTTTCAGTG GGAGGGACAG GGGGGGTTT GGGGAGTGGC TAACCTCAG AAGCTGCATA					
15	250	260	270	280	290	300
	TAAGCAGCG CTTTCGTCTT GTACCGGTTC TCGGTAGAG GACCAGGTCT GAGCCCGGGA					
	310	320	330	340	350	360
	GCTCCCTGGC CTCTAGCTGA ACCCGCTCGT TAACGCTCAA TAAAGCTTGC CTTGAGTGAG					

20

A.

4. The retrovirus of any of claims 1 to 3 characterized in that its RNA virtually hybridizes neither with the Env gene and the LTR close to it, in particular not with the nucleotide sequence 8352-9538 of HIV-1, nor with the sequences of the Pol region of the HIV-1 genome under stringent conditions.

25

5. A composition comprising at least one antigen, in particular a protein or glycoprotein of HIV-3 retrovirus of any of claims 1 to 4.

30

6. The composition of claim 5 characterized by containing a total extract or lysate of said retrovirus.

35

000000-000000

1 7. The composition of claim 5, characterized by containing
at least one of the internal core proteins of said
retrovirus, in particular p12, p16 or p26 having apparent
5 molecular weights in the order of 12,000, 16,000 and 26,000
respectively.

8. The composition of claim 5, characterized by containing
at least one of the envelope proteins of said retrovirus, in
10 particular gp41 or gp120 having apparent molecular weights
in the order of 40,000-45,000 and 120,000 respectively.

9. An antigen providing a single band in polyacrylamide gel
electrophoresis, said antigen comprising, in common with one
15 of the purified antigens of HIV-3 retrovirus, an epitope
that is recognized by serum of a patient carrying anti-HIV-3
antibodies.

10. A purified antigen having the immunological
20 characteristics of one of the following proteins or
glycoproteins of HIV-3: p12, p16, p26, gp41 and gp120.

11. The antigen of claim 10 having the aminoacid sequence,
or a part of said sequence, of the p12 protein obtained by
25 subjecting the protein mixture produced by HIV-3 to gel
electrophoresis and isolating the p12 protein in a manner
known per se.

12. The antigen of claim 10 having the aminoacid sequence,
or a part of said sequence, of the p16 protein obtained by
30 subjecting the protein mixture produced by HIV-3 to gel
electrophoresis and isolating the p16 protein in a manner
known per se.

13. The antigen of claim 10 having the aminoacid sequence,
or a part of said sequence, of the p26 protein obtained by
35 subjecting the protein mixture produced by HIV-3 to gel
electrophoresis and isolating the p26 protein in a manner
known per se.

000000-000000

1 14. The antigen of claim 10 having the aminoacid sequence,
or a part of said sequence, of thegp41 protein obtained by
subjecting the protein mixture produced by HIV-3 to gel
5 electrophoresis and isolating the gp41 protein in a manner
known per se.

15. The antigen of claim 10 having the aminoacid sequence,
or a part of said sequence, of thegp120 protein obtained by
10 subjecting the protein mixture produced by HIV-3 to gel
electrophoresis and isolating the gp120 protein in a manner
known per se.

16. A method for the detection of antibodies against HIV-3
15 retrovirus in a biological liquid, such as a serum or spinal
fluid, in particular for the diagnosis of a potential or
existing ARC or AIDS caused by said HIV-3 retrovirus,
characterized by contacting body fluid of a person to be
diagnosed with a composition of any of claims 5 to 8 or with
20 an antigen of any claims 9 to 15 and detecting the
immunological conjugate formed between said anti-HIV-3
antibodies and the antigen(s) used.

17. The method of claim 16, characterized in that said
25 detection of said immunological conjugate is achieved by
reacting said immunological conjugate with a labeled reagent
selected from antihuman immunoglobulin-antibodies or
bacterial A protein or G protein and detecting the complex
formed between said conjugate and said reagent.

30 18. A kit for the detection of anti-HIV-3-antibodies in a
biological fluid, comprising

- a composition as defined in any of claims 5 to 8 or an
35 antigen as defined in any of claim 9 to 15, and
- means for detecting the immunological complex formed.

1 19. The kit of claim 18, characterized in that said means
for detecting said immunological complex comprise antihuman
immunoglobulin(s) or protein A and means for detecting the
5 complex formed between the anti-HIV-3 antibodies contained
in the detected immunological conjugate.

10 20. An immunogenic composition containing an envelope
glycoprotein of HIV-3 retrovirus, in particular gp41 or
pgl20, or a part of said glycoprotein, in combination with a
pharmaceutically acceptable vehicle suitable for the
constitution of vaccines effective against HIV-3.

15 21. The composition of claim 20, characterized by containing
at least part of a glycoprotein comprising the protein
backbone of the envelope protein. or a part thereof, as
defined in any of claims 14 to 15.

20 22. Monoclonal antibodies characterized by their ability to
specifically recognize one of the antigens as defined in any
of claims 11 to 15 in particular monoclonal antibodies
specifically raised against said antigens.

25 23. The secreting hybridomas of the monoclonal antibodies of
claim 22.

24. Nucleic acids, optionally labeled, derived in part at
least of RNA of HIV-3 retrovirus or of variants thereof.

30 25. The nucleic acid of claim 24, characterized by
containing at least part of the cDNA corresponding with the
entire genomic RNA of HIV-3 retrovirus.

35 26. The nucleic acid of claim 24 containing the nucleotide
sequence as identified in claim 3.

27. The nucleic acids of claim 24 characterized by
containing nucleotide sequences coding for at least part of
the aminoacid sequences of proteins as defined in any of
claims 11 to 13.

28. The nucleic acids of claim 24, characterized by containing nucleotide sequences coding for at least part of the amino acid sequences of glycoproteins as defined in any of claims 14 to 15.

29. The nucleic acids of any of claims 24 to 28, characterized by being formed into a recombinant nucleic acid comprising a nucleic acid from a vector having said cDNA, or a part of said cDNA, inserted therein.

30. The recombinant nucleic acid of claim 29 characterized by being labeled.

31. A process for the detection of HIV-3 retrovirus or of its RNA in a biological liquid or tissue, characterized by contacting nucleic acids contained in said biological liquid or tissue with a probe containing a nucleic acid according to any of claims 25 to 30 under stringent hybridization conditions, washing the hybrid formed with a solution preserving said stringent conditions, and detecting the hybrid formed.

32. A process for the production of HIV-3 retrovirus characterized by culturing human T4 lymphocytes, or permanent cell lines derived therefrom carrying the T4 phenotype, with lymphocytes or cell lines that have previously been infected with an isolate of HIV-3 retrovirus, as well as recovering and purifying the retrovirus from the culture medium.

33. A process for the production of antigens of HIV-3 retrovirus, characterized by lysing the retrovirus and recovering the lysate containing said antigens.

35

1

34. A process for the production of any of the proteins or glycoproteins p12, p16, p26, gp41 and gp120 as defined hereinbefore, or of a part thereof, characterized by inserting the corresponding nucleic acid sequence in an expression vector, transforming a host with said vector, culturing the transformed host as well as recovering and purifying the expressed protein.

5

10

35. A process for the production of a hybridization probe for the detection of the RNA of HIV-3 retrovirus, characterized by inserting a DNA sequence, particularly of any of claims 24 to 29 in a cloning vector by in vitro recombination, cloning the modified vector obtained in a suitable cellular host, and recovering the hybridization probe.

15

20

36. A method for detecting antigen of HIV-3, characterized by coating a surface with an immunoglobulin fraction raised against HIV-3, bringing a body or culture fluid to be analyzed into contact with the immunoglobulins, and detecting the complex formed between the immunoglobulins and the antigen.

25

30

ADD
A'4

35

000000-000000